# UPTAKE AND FATE OF EXOGENOUS CELLULAR DNA

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# Factors to be considered

1. Nature of residual DNA
2. Uptake efficiency of DNA by cells
3. Survival of DNA within cells
4. Efficiency of recombination events
5. Complex nature of tumorigenesis

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# Exposure to DNA

1. DNA in food
2. Infectious agents
3. Fetal DNA in maternal blood
4. Plants - pollen
5. Gene Therapy
6. Biologicals / Vaccines

# How do cells deal with DNA to which they are exposed?

**How does it get into cells?**

**1. Endocytosis – (pinocytosis)**

**What happens to the internalised vesicles?**

**2. Targeted to lysosomes**

**3. Endosome escape**

**4. Re-cycling to plasma membrane**

**5. Nuclear targeting and handling**

## In context of this session: therefore 2 main questions

1. Can residual cellular DNA get into cells?
   (without viral elements)

2. If yes – can it escape destruction, integrate into genome and cause adverse effects?

# Faced with a paradox

**1. FOR SAFETY ISSUES**

**How to minimise the risk of transformation events arising as a result of residual (contaminant) DNA**

**e.g. specific vaccine products.**

**2. FOR GENE THERAPY**

**How to maximise transfection/infection efficiency of somatic cells and DNA stability of genetic material in question**

**- for sake of successful gene therapy**

# Mammalian cellular uptake of DNA

## Inefficient process - requires facilitator

1. DEAE Dextran

2. Calcium Phosphate

Uptake enhanced by inclusion of carrier DNA (high MW)

3. Lipofection

4. Electroporation

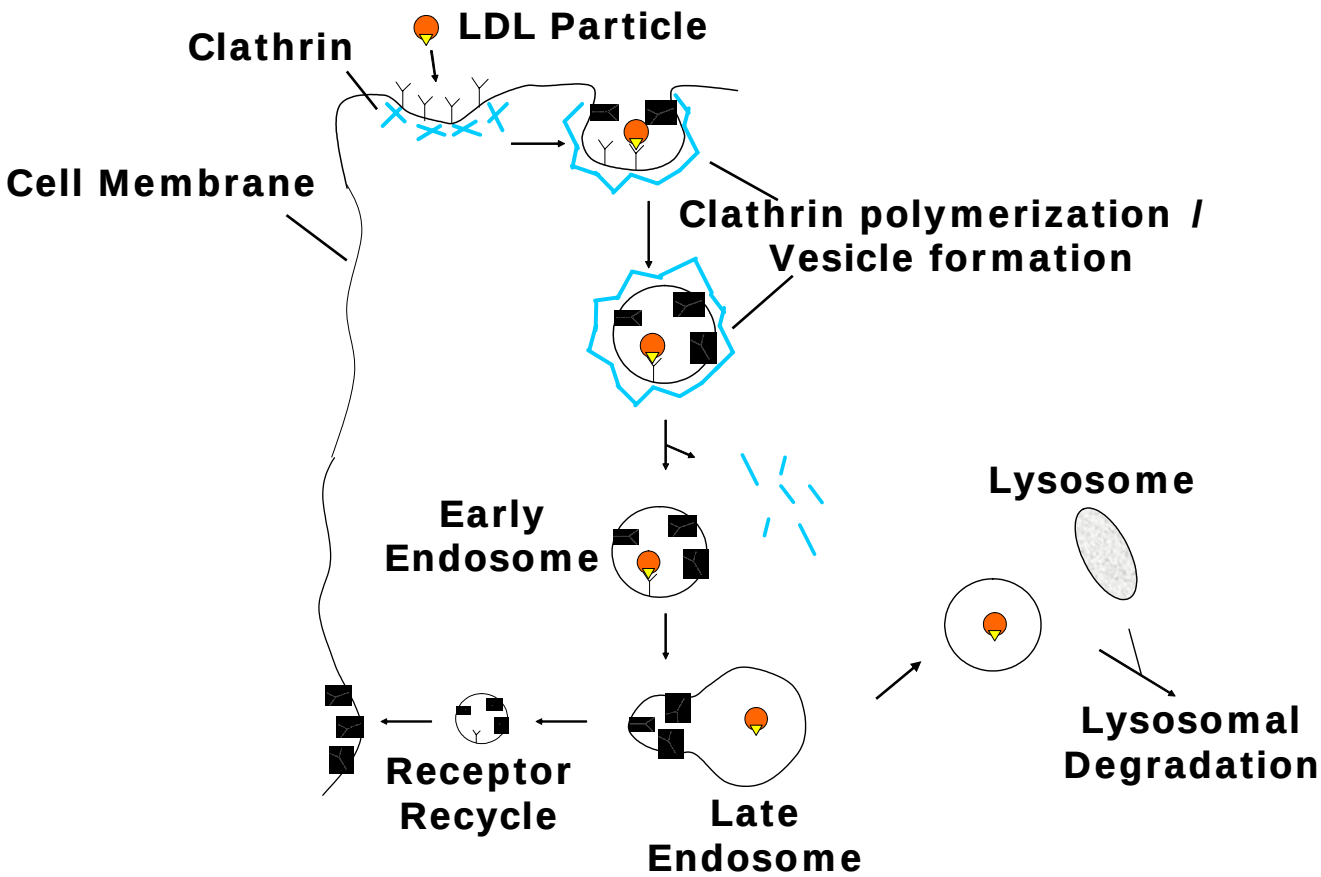
# Mammalian cellular uptake of DNA

**These methodologies are all extremely inefficient**
**70's and 80's**
**In reality very difficult to get cellular DNA into cells**

**Nowadays – 1990's / 00's**
**viral vectors**

- adenovirus
- retrovirus
- lentivirus

# Receptor-Mediated Endocytosis




Classic Example: Low-Density Lipoprotein (LDL)

## Surprisingly little known about nucleases wich degrade DNA - other than in lysosomes

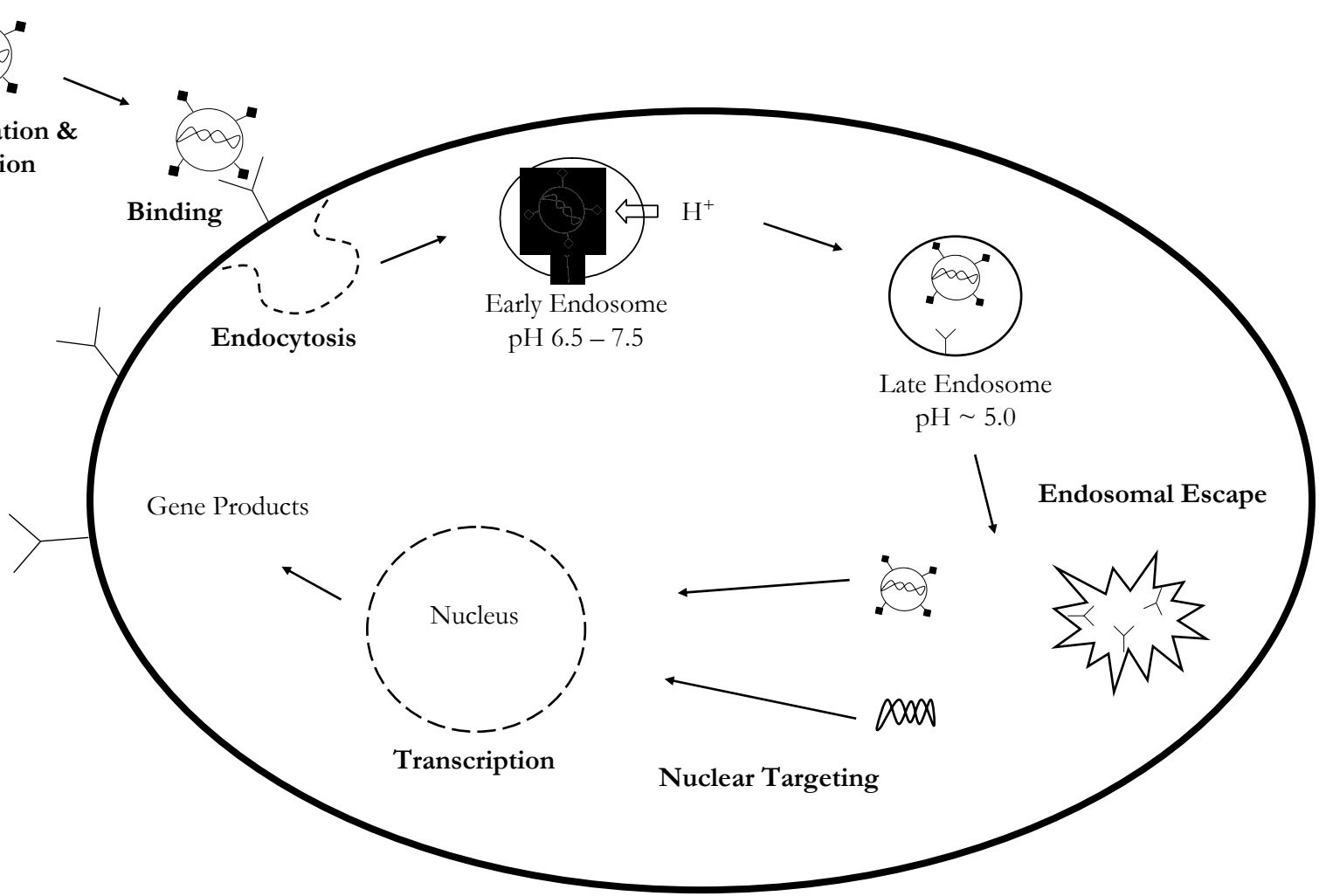
**NUCLEASES**

**Recent Review Article :**

**Numerous events ascribed to nucleases**

**RNAases, DNAases**

**Replication, recombination, repair,**




Designing Gene Delivery Systems

Target lysosomes – adjust pH to render nucleases less active

# Mammalian cellular uptake of DNA

1970's and 80's Van der Eb, Doerfler, Strain

Evidence that radio-labelled exogenous DNA handled with efficiency (degraded) by cells. However, only small percentage need be retained and fate under scrutiny

Fundamental :- in the absence of viral vectors to get DNA into cells, what the old studies showed is that equally in the absence of 'facilitators' very difficult to get DNA into cells and that which does has little likelihood of surviving.

# Howard Temin

**Reported risk estimates of DNA from cells without active oncogenes or with or the possibility of inactivating tumour suppressor genes**

**W Doerfler P Meulien R Kurth**

**Maximum cumulative probability of adverse effects estimated at $10^{-16}$ to $10^{-19}$ per DNA molecule from a cell without activated proto-oncogenes or viral oncogenes**

**$10^{-17}$ with DNA from cell with an active proto-oncogene or viral oncogene**

**1990 – 2001 literature**

# Probability of an oncogenic or inf. Event @ 100 pg DNA

- 1984 FDA/NIAID Workshop — Rough guess
  - 10 pg / dose
- 1986 WHO Study Group — $1 / 2 \times 10^{10}$
  - 100 pg / dose
- 1987 Regan — $1 \times 10^{10}$
- 1990 Temin — $1 / 10^{12}$
- 1995 Kurth — $1 / 10^{12}$
- 1997 Dortant et al — $1 / 5 \times 10^{8}$
- 1999 Krause / Lewis — $1 / 4 \times 10^{9}$

## Other recent studies

# FATE OF DNA IN GI-TRACT EPITHELIUM

**Palka-Santini et al (2003) - Doerfler group**

**"The gastrointestinal tract as the portal of entry for foreign macromolecules: fate of DNA and proteins"**

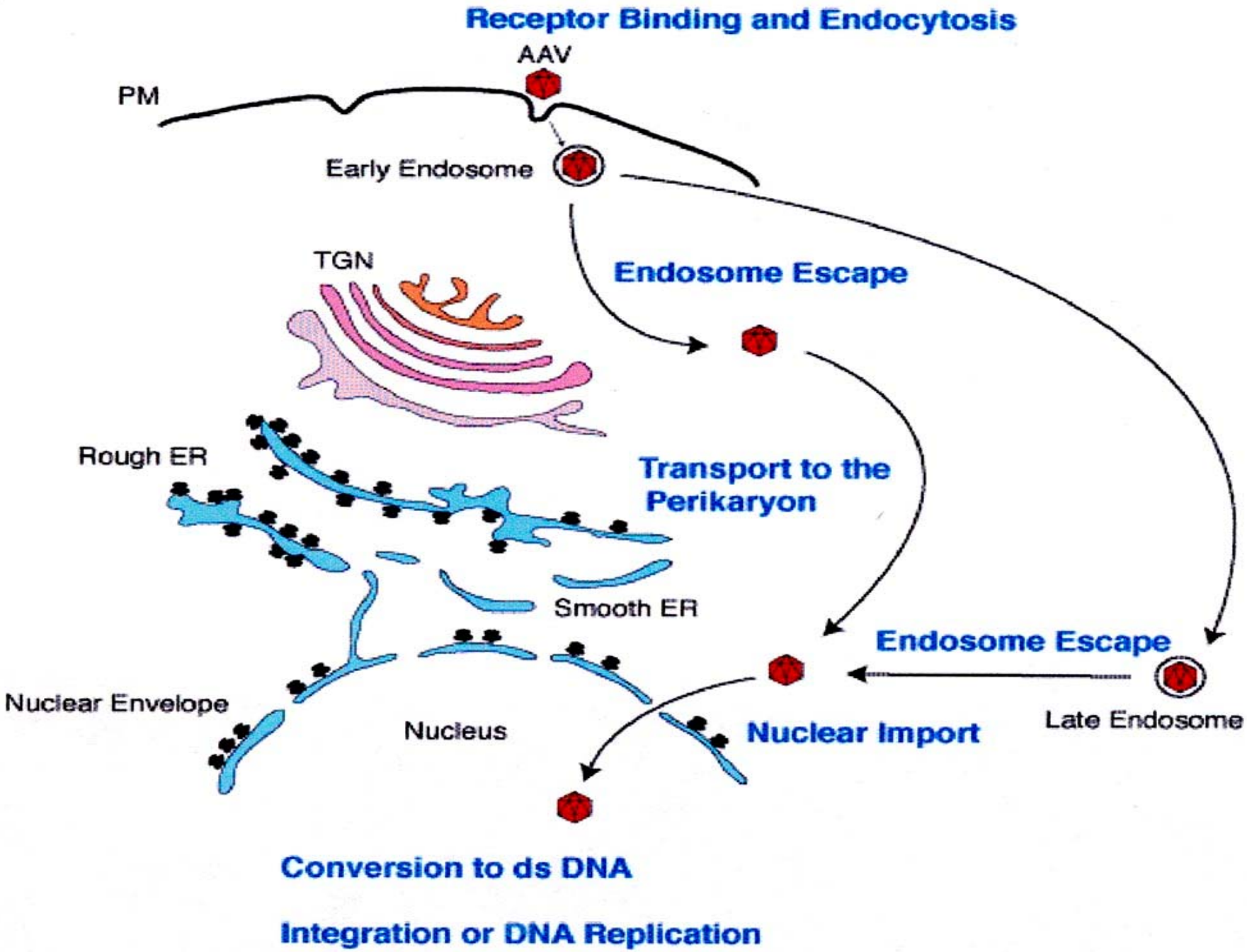
**Notably persistence in cells not detectable beyond day 1**

**Forsman et al (2003)**

**"Uptake of amplifiable fragments of retrotransposon DNA from the human alimentary tract"**

**Elaborate mechanisms for inactivating foreign DNA that traverses the mucosal epithelium lining the intestine**

**DNA fragments found in plasma but short lived**


Receptor Binding and Endocytosis
AAV
PM
Early Endosome
TGN
Endosome Escape
Rough ER
Transport to the Perikaryon
Smooth ER
Endosome Escape
Nuclear Envelope
Nucleus
Nuclear Import
Late Endosome
Conversion to ds DNA
Integration or DNA Replication

# ENDOCYTOSIS

Inward vesiculation driven by clathrin

Outward vesiculation now better understood

E-class proteins first described in yeast
(vacular protein-sorting (Vps) components)

Viruses may subvert cells' machinery to escape

View that endocytosis leads inexorably to destruction in lysosomes no longer tenable

Endocytic compartments can contribute to recycling, storage, regulated secretion and membrane repair

also now evidence that some viruses can bud directly into endocytic vesicles

some viruses may use cell's ESCRT machinery

(endosomal sorting complex required for transport)

# IN CONCLUSION

Theoretical risk will always exist

Without ‘assistance’ DNA does not enter cells with ease

Nuclease-mediated destruction

‘Normal‘ cellular DNA poses minute risk

Tumour cell derived DNA theoretically greater potential risk but remains small

If any risk (however small) does this outweigh the benefit?